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PROTECTION OF PLANTS FROM IONIZING RADIATION

- USSR -

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PROTECTION OF PLANTS FROM IONIZING RADIATION

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[Following is a translation of an article by L. P. Breslavets and Z. F. Mileshko in the Russian-language periodical Priroda (Nature), Moscow, No. 5, May 1960, pages 50-53.]

The radioactivity of the air and the water is increasing with each passing year. According to data provided by Japanese meteorological stations, the radioactivity of rain water in 1958 increased to twice that of 1957. This forces one to think seriously about the protection of plants, animals, and human beings from the harmful effect of radiations.

What Have the Experiments Proved?

An infinitely large number of experiments have been carried out on the higher animals with the aim of finding chemical substances whose administration into the organism could "protect" them from being affected. But the word "protection" is used in various senses in scientific literature. As applied to mice, rats, guinea pigs, and other small animals it usually means the ability (after a particular dosage of radiation) of a greater percentage of the animals to remain alive or to withstand a greater dose than without the application of the protective substances. The data is usually based on the animals that have remained alive 30 days after radiation. It would be incorrect to think that animals that have remained alive that long are normal in all respects. However, with a certain limitation it may be felt that animals to which certain "protective" substances have been administered withstand a stronger radiation than those which have not received them. Cysteinamine is used most widely for purposes of protection. This substance proved to be especially effective in experiments with bacteria, for example Escherichia coli. After being treated with cysteinamine they withstood radiation 12 times greater than without it.

In the experiments of A. Forssberg and N. Nyuborn (Sweden, 1953), other substances related to cysteinamine protected onion roots from the effect of x-rays. It must be considered that the protective substance can be used not only during radiation, but also before and after it. In our experiments (1954) we took the

first path: one batch of rye seeds was soaked in ribose nucleic acid, another in omaine, and a third in tap water. Forty-eight hours later, all three batches were simultaneously given a dosage of 2000 roentgens, with the exception of half the seeds which had been soaked in water and which served as the control. The seeds which had been soaked in water and exposed to radiation germinated very slowly and died within three weeks. The rye seeds that, prior to radiation, had been soaked in a 2% solution of RNA [ribose nucleic acid] produced coleoptiles (vaginal leaflets) of the same size as those that were not exposed to radiation, but their roots did not undergo any protective action, and for that reason the sprouts did not live long. Among the radiated sprouts that developed from the seeds that had been soaked in omaine, the roots reached the same length as among the control plants, but the coleoptiles did not feel the effect of any protective action, and these plants also proved to be inferior and died soon.

By changing the concentration of the protective substances and by combining them with one another, we obviously could have achieved a situation in which the sprouts soaked in them would be protected from radiation. N. V. Luchnik (1958) used the action of protective substances after radiation. By preliminary experiments he established that peas soaked for 24 hours stop developing if they are given a radiation dosage of 600 roentgens. But if, after radiation, the pea seeds were given yeast extracts, they developed normally. These research works showed that the RNA was the effective beginning in the extracts. It produced the same protective effect as the yeast extracts.

Thus, by using RNA before radiation, as we had done, or after radiation, as was done in N. V. Luchnik's experiments, the plant can be protected from the harmful effect of radiation.

We scarcely think that chemical substances can find practical application for protecting plants from ionizing radiation. However, the experiments that were carried out provide valuable indications, even now, of the mechanism involved in the action of ionizing radiation, and they may in time lead to practical results.

The idea that such protective measures are, for the time being, applicable only under conditions of the laboratory or of a small experiment forced us to turn to another method of protecting plants. As far back as 1929, L. Dzh. Stadler (United States) showed that plant species possessing a large number of chromosome sets are less sensitive to the action of radiation than plants with a limited set of them. Many subsequent works confirmed that assumption. However, in most instances comparison was made of the action of radiation upon various species or even various genera of one and the same family. For example, in the research carried out by Dzh. Kh. Mak-Ki (United States, 1954), comparison was made between barley species having few chromosomes and wheat species

having many chromosomes. K. Mikel'sen and K. Aastvort (Norway, 1957) carried out experiments involving the action of two types of radiation -- by various neutrons and the chronic action of gamma-rays -- upon the growth and fertility of barley having 14 diploid chromosomes in the cells and oats with 42 diploid chromosomes. No special differences were observed in the reaction of the barley and the oats to the gamma-rays, but the barley proved to be three times more sensitive to the various neutrons than the oats. Nevertheless one must not forget that the objects of research were different species, and that fact might have introduced specific factors into the experiment. The purity of the experiment would have been significantly gained if comparison had been made of the action of radiation upon the plants with an ordinary number of chromosomes and upon plants with a multiplied set of them. This kind of experiment was carried out for the first time by A. Myuntsing (Sweden, 1941). By observing the growth and development of diploid and tetraploid plants of barley that had been grown from seeds exposed to x-rays, he showed that the tetraploids are considerably more resistant to the action of radiation than diploids. The same results were obtained by V. V. Mansurova, V. V. Sakharov, and V. V. Khvostova (1958), who gave dosages of radiation to diploid and tetraploid seeds of buckwheat and millet.

Do Polyploids Possess Greater Resistance?

The authors of this article (1956) tested the action of two types of radiation--neutrons and gamma-rays--upon rye diploids and tetraploids, with the aim of determining the effect of radiation in the ontogenesis (period of individual development) of the plants. Only a small number of the seeds that were exposed to radiation were planted in dirt-filled boxes and kept in the greenhouse, and the others were sown on small plots in the field (it is more convenient to observe the action of these two types of radiation separately). The neutrons were administered at an identical stream ($4.65 \cdot 10^8$ cm/sec), but with varying duration: 30 minutes, one hour, 2, 3, 6, and 9 hours. Thirty seeds from each batch of radiated seeds and from the control were planted in the dirt-filled boxes in the greenhouse, where it was convenient to observe their development. The fourth day after planting, all the control seeds, both diploid and tetraploid, germinated, and on the sixth day all the diploids and 24 of the tetraploids produced their first leaflets. However, the sprouts from among the seeds that had been radiated by the neutrons acted differently, depending upon the duration of the radiation. With a high dosage of radiation, the protective action of the tetraploid structure had its effect: with radiation for one hour, only six diploid sprouts produced leaflets; the tetraploids had 17. After two hours of radiation, not a single diploid

sprout formed leaflets, but seven of the tetraploids formed them. These observations show that a doubled number of chromosomes in the set makes the germ of the seeds less sensitive to radiation. The considerably greater protective action of polyploid structure manifested itself on the initial growth of the irradiated plants.

As shown by the measurements, the control tetraploid sprouts first develop more slowly than the control diploids, then catch up with them, and by the eighteenth day have greatly outstripped them. With the action of the first dose of radiation (30 minutes), a lesser radiosensitivity of the tetraploid sprouts begins to be felt. This difference manifests itself still more considerably when the radiation lasts one to two hours. With higher doses, both types of sprouts suffer almost identically from the action of neutrons, and, finally, when the radiation lasts 9 hours, they all die by the eighth day after planting. By the end of the vegetation period, all the irradiated sprouts died under greenhouse conditions.

On the basis of these measurements, a curve was constructed which shows the difference in the sensitivity of diploid and tetraploid sprouts to the action of neutrons (Figure 1).

The irradiated seeds that were planted in the field continued to vegetate, and some of the plants, even those that had received a high dose of neutrons, continued to grow to maturity. This made it possible to determine the different state of the pollen seeds in the diploids and tetraploids with respect to their fertility time. The criteria used were the separation of the plasma from the walls of the pollen seeds, and the iodine reaction. In normal pollen seeds of rye, the plasma adheres tightly to the walls, and the seeds contain such a large number of starch grains that when they are carried over into drops of iodine in potassium iodide they appear to be black.

We see a completely different picture when plants are irradiated with neutrons. The plasm in their pollen seeds is far away from the walls, it is crinkled, and, when the radiation is prolonged, forms lumps. Pollen seeds of this type do not have an iodine reaction, but remain colored yellow. The pollen seeds formed in plants that received the highest irradiation dose have an even blue coloration of their content by black sudan, which attests to the formation of fat in them, instead of starch. These reactions of the pollen seeds to radiation were considerably more clearly expressed in the diploid plants.

Consequently, the protective action of tetraploid structure manifests itself not only at first stages of development, but also through the entire ontogenesis.

In the experiments by K. Mikel'sen and K. Aasvort (Norway), the action of gamma-rays was distinct from the action of neutrons. Our experiments led to different results. For an exact comparison of the effect of the two types of radiation, the experiments with

the gamma-rays were set up in completely identical conditions, and at the same time as the experiments for studying the effect of neutrons. The seeds irradiated with gamma-ray doses that were high for rye were planted in dirt-filled boxes in the greenhouse. The sprouts of the irradiated and the control seeds were measured three times. The measurements showed what a powerful means of protection the tetraploid state of the plant can be. This becomes especially graphic when the dose is as high as 10,000 roentgens; at the third measurement, the diploid sprouts scarcely have reached a third of the height of the control plants, and the height of the tetraploid plants is close to that of the normal plants. Still higher doses of gamma-rays proved to be 3 times less harmful for tetraploids than for the diploids. Even plants that received 30,000 roentgens were able to remain alive on the field for a comparatively long time, and one of them reached maturity. The protective action of the tetraploid structure can be seen especially graphically on the curve shown (Figure 2).

Both for the plants irradiated with neutrons and for those that grew from seeds that had received various doses of gamma-rays, determination was made of the number of nonviable pollen seeds and the number of seeds producing the iodine reaction. As far as can be judged on the basis of the behavior of the pollen seeds, the protective action of their tetraploid structure also manifests itself throughout their individual development (ontogenesis). With action of 20,000 roentgens it manifests itself in a higher percentage of nonviable pollen seeds and in a larger percentage of seeds that do not produce the iodine reaction.

Sensitivity of Insects

But vegetable organisms are not the only ones that can be protected from radiations. As can be seen from the experiments of A. R. Whiting and Ch. Bostian (United States, 1931), haploid male larvae of the ichneumon fly Habrobracon juglans are considerably more sensitive to x-rays than female diploid larvae. This difference could be prescribed to the differential sensitivity of the males and females, if the later experiments of A. M. Clark and K. J. Mitchell (1939) and A. M. Clark and Ye. M. Kelly (United States, 1950) which were carried out with haploid and diploid males of the same insect had not confirmed the greater resistance rate of the diploid organisms. This is proved by experiments of B. L. Astaurov and N. P. Tul'tseva (1958).

Experiments with the radiation of diploid and polyploid plants and animals indicate the communality of their reaction to radiation depending upon the degree of polyploid state. But this method of protection will probably find its greatest application among plants, since among animals the obtaining of artificial

polyploids encounters great difficulties and is limited to a small number of individuals, whereas for plants the multiplication of polyploid forms can be limitless.

The data obtained in this direction is, for the time being, the result of applying large doses of radiation, and the reactions described in these works are very rough. But one must hope that a method will be found for ascertaining the various reactions to ionizing radiation among the diploids and polyploids with lower doses, making use of biochemical and physical indexes, and also by the use of the fluoroscopic and electronic microscope. This will open up new paths for the protection of plants from the increasing ionization of the air.

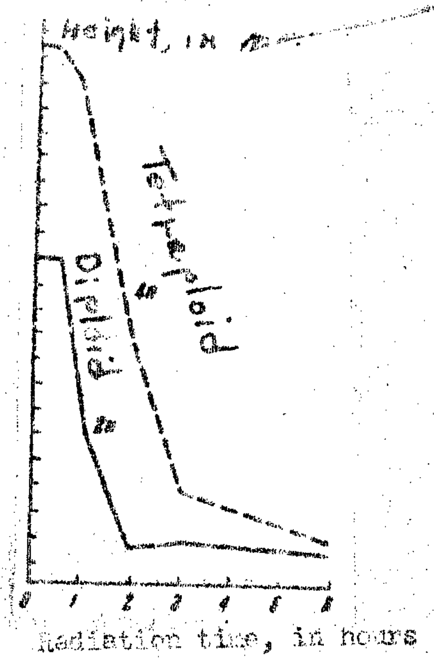


Figure 1. Height (in millimeters) of diploid and tetraploid rye sprouts that received different doses of radiation by neutrons (on the eighteenth day after sprouting)

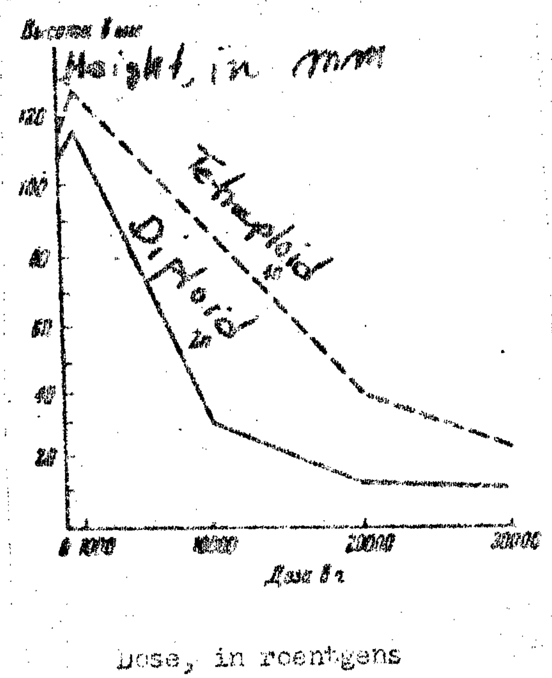
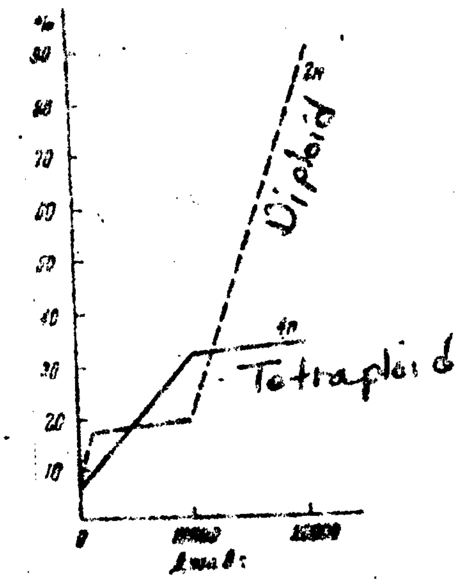


Figure 2. Height of diploid and tetraploid rye sprouts that received different doses of gamma-rays



Dose, in roentgens

Figure 3. Absence of iodine reaction in the pollen seeds of diploid and tetraploid rye irradiated with different doses of gamma-rays.

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